# L. A STUDY OF THE BLOOD LIPOIDS AND BLOOD PROTEIN IN CANADIAN EASTERN ARCTIC ESKIMOS

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THE data reported here were part of a clinical and metabolic study of Eskimos in 1935, during the Canadian Government Eastern Arctic Patrol on the R.M.S. Nascopie. They included Eskimos in Hudson Bay and Strait, the more northerly parts of Baffin Island and in Devon and Ellesmere Islands, as far north as 76° 12′ latitude—828 nautical miles from the Pole. The clinical and other metabolic data were reported elsewhere [Rabinowitch, 1936; Rabinowitch & Smith, 1936]. This communication is concerned with blood lipoids only.

#### METHODS OF INVESTIGATION

#### Selection of subjects

To determine the effects of the different dietary habits, the subjects were divided into two groups (see p. 345).

#### Materials collected

The data included samples of blood collected (a) at random and (b) after fat meals which consisted of 200 ml. of soya bean oil.

#### Chemical analyses

None of the tests was completed during the voyage. The work then was confined to collection of the blood samples and their necessary treatment to preserve the different constituents to be examined. All analyses were made on oxalated plasma. With few exceptions, all the samples were collected by one of the writers (I. M. R.)¹ and subjected to the same preliminary preparation. In each case, the blood was centrifuged as soon as possible after it had been obtained and a known amount of the separated plasma was immediately added, drop by drop, with constant shaking, to a flask which contained about 50 ml. of Bloor's [1916] alcohol-ether mixture. The flask was then well stoppered and the mixture kept cold and in the dark during the entire voyage and then kept in the refrigerator of the laboratory until the analyses were made. Bloor [1916] has shown that such mixtures keep unchanged for a considerable period under these conditions. In some cases, owing to tide conditions, the bloods could not be centrifuged immediately, since all the laboratory work had to be done on the

<sup>1</sup> A portion of the material was collected by Dr C. C. Birchard in Hudson Strait and on the Quebec shores of Hudson Bay.

ship. However, unlike sodium chloride and other blood constituents, lipoids are not appreciably affected in their relative distribution by contact of cells with plasma of shed blood for a few hours only.

Extraction process. The total contents of each flask, including the protein precipitate, were transferred to a 125 ml. Erlenmeyer flask. The original container and its stopper were then rinsed repeatedly with the above-mentioned alcoholether solution and the rinsings added to the extract to a total volume of 80 ml. Any small masses of precipitate were divided very finely with a glass rod. The extract so obtained was then filtered and the filtrate was diluted to 100 ml. with the alcohol-ether solution as suggested by Boyd [1936]. Proof that the losses with this method were negligible was found in repeated control experiments with other samples of plasma and other extraction procedures.

Methods. All analyses were made by one of the writers (A. C. C.). Total fatty acids, total and free cholesterol and phospholipin were determined by Boyd's [1931; 1933; 1935] modifications of the micro-oxidative methods of Bloor [1928; 1929] and Yasuda [1931]. According to repeated recovery experiments, the errors of these procedures were found to be within the limits reported by their authors.

Standards. Boyd [1933; 1934; 1935] has recorded three separate sets of normal values. Two of these [1933; 1934] were, however, obtained in women only. The last set [1935] includes both males and females and the figures were obtained with the above-mentioned methods. They are, therefore, comparable with our own and were used as standards (Table I).

Table I. Concentrations of plasma lipoids in normal individuals

	Total lipoids	Neutral fat	Total fatty acids		Ester chole- sterol			$\frac{\text{Ester}}{\text{cholesterol}}$ $\frac{\text{cholesterol}}{\text{cholesterol}}$	Total
Maximum	718	205	429	220	157	63	188	0.71	1.13
Minimum	506	146	301	141	101	41	151	0.68	0.97
Average mean	595	150	349	177	124	53	185	0.70	1.04

#### DISCUSSION OF RESULTS

In Table II are recorded the combined results obtained in 27 Eskimos. These show that, compared with the standard (Table I), the average concentration of total lipoids was low, in spite of the fact that the diets of 20 of these 27 Eskimos consisted essentially of protein and fat. The average concentrations of total free and ester cholesterol, of phospholipins and the average ratio of ester to total cholesterol were also lower than those found with similar methods amongst civilized peoples; and that the mean in each case is not accidental and the result of isolated low values, but a true indication of the mode, is shown from the number of individuals in whom low values were found. Thus, by comparing Tables I and II, it will be noted that 11 of the 27 values of total lipoids—an incidence of 41·1 %—were not only lower than the average, but lower than the minimum concentration found amongst peoples elsewhere. Values lower than the minimum standard were also found in 14 of the 27 total cholesterol determinations—an incidence of 51.8%; in 15 of the ester cholesterol values—an incidence of 55.5%; in 11 of the phospholipin values—an incidence of 41.1% and in 15 of the ratios of ester to total cholesterol—an incidence of 55.5 %. Higher average values were found in the case of neutral fat and the ratio of

Table II. Showing concentrations of plasma lipoids in Canadian Eastern Arctic Eskimos

(mg. per 100 ml.)										
		(—8. per 200 mil)							Ester	Phospho-
				Total	Total	Ester	Free	Phos-	cholesterol	lipins
		Total	Neutral	fatty	chole-	chole-	chole-	pho-	Total	Total
No.		lipoids	$\mathbf{fat}$	acids	sterol	sterol	sterol	lipins	cholesterol	cholesterol
1	Hudson	627	348	447	136	97	39	81	0.76	0.60
<b>2</b>	Bay and	357	92	213	93	52	41	137	0.56	1.47
3	Strait	491	271	340	122	<b>74</b>	48	47	0.63	0.40
4		551	225	355	147	102	45	111	0.69	0.76
5		548	168	336	153	111	<b>42</b>	152	0.72	0.99
6		582	25	286	<b>222</b>	168	54	223	0.76	1.00
7		393	106	233	116	<b>74</b>	<b>42</b>	121	0.62	1.05
8	Baffin	503	58	271	164	127	37	196	0.77	1.13
9	$\mathbf{Island}$	<b>554</b>	187	343	149	92	57	156	0.62	1.05
10		<b>664</b>	368	<b>494</b>	96	50	46	166	0.52	1.72
11		718	207	431	209	149	60	202	0.71	0.97
12		505	116	306	137	97	40	187	0.71	1.36
13		<b>437</b>	165	287	100	71	29	124	0.71	1.24
14		753	345	514	164	108	<b>56</b>	172	0.67	1.05
15		593	112	349	158	104	54	235	0.66	1.42
16		500	179	318	128	86	<b>42</b>	135	0.67	1.05
17		765	270	<b>495</b>	179	122	<b>56</b>	234	0.69	1.30
18		565	155	336	166	117	49	166	0.70	1.00
19		463	186	305	109	69	40	122	0.63	$1 \cdot 12$
20		395	172	256	107	72	35	68	0.67	0.73
21		558	199	<b>350</b>	152	103	49	138	0.66	0.90
22	Devon	570	<b>224</b>	377	133	90	43	153	0.67	1.15
23	$\mathbf{Island}$	624	266	417	146	106	40	141	0.72	0.97
24		703	204	<b>465</b>	116	69	47	337	0.58	2.87
25		481	156	302	115	62	53	168	0.54	1.46
26		470	65	252	158	113	<b>45</b>	171	0.71	1.82
27		613	164	388	125	75	50	274	0.67	$2 \cdot 19$

phospholipin to total cholesterol and that here also the mean was a true indication of the mode is shown by the fact that of the 27 determinations of neutral fat, concentrations greater than the maximum value of the standards were found in 9—an incidence of  $33\cdot3\%$ , and of the ratios of phospholipins to total cholesterol, 11—an incidence of  $41\cdot1\%$ —were higher than the normal maximum of peoples elsewhere.

In order to determine the effects, if any, of the dietary habits of these natives, the data, as stated, were divided into two groups, namely, (a) those obtained in Hudson Bay and Strait amongst natives who live, to an appreciable extent, upon mixed diets, and (b) those obtained in Baffin and Devon Islands

Table III. Showing average concentrations of plasma lipoids in normal civilized peoples and in Canadian Eastern Arctic Eskimos on different diets

			T7-4	T01 . 1					
	Total lipoids	Neutral fat	Total fatty acids	Total chole- sterol	Ester chole- sterol	Free chole- sterol	Phos- pho- lipins	$\frac{\text{Ester}}{\text{cholesterol}}$	Phospho- lipins Total cholesterol
Standards*	595	150	349	177	124	53	185	0.70	1.04
Eskimos:									
Whole group	555	186	351	141	98	46	164	0.67	1.21
Mixed diet	507	176	316	141	97	44	124	0.68	0.90
Meat-eaters	<b>572</b>	190	363	141	99	46	178	0.66	1.32
			*	[Boyd,	1935].				

amongst natives whose diets, except for about 2 months in the year, consist of the natural foods of their environment (seal, whale, narwhal, walrus, etc.). A summary of this division of the data is recorded in Table III in which are also recorded, for comparative purposes, the average values found with the same technique amongst civilized peoples. It will be noted that the average concentrations of total lipoids, neutral fat, total fatty acids and phospholipins and the average ratio of phospholipins to total cholesterol were higher amongst the meat-eaters than amongst those whose diets, in addition to meats, consisted also of appreciable quantities of carbohydrates (flour etc.).

## Effects of ingestion of fat

In Table IV are recorded two experiments in which the concentrations of the different plasma lipoids were determined before and after administration of 200 ml. soya bean oil. Both subjects were carefully examined and were found

Table IV. Showing effects of ingestion of soya bean oil upon concentrations of plasma lipoids in Canadian Eastern Arctic Eskimos

(mg. per 100 ml.)									Phospho-		
Time	Total lipoids	Neutral fat	Total fatty acids	Total chole- sterol	Ester chole- sterol	Free chole- sterol	Phos- pho- lipins	Ester cholesterol Total cholesterol			
Subject A											
Basal 1 hour after 2 hours after	470 397 	68 53 —	$252 \\ 206 \\ 201$	158 149 128	113 103 89	45 41 39	171 131	0·71 0·71 0·69	1·08 0·89		
3 hours after	444	131	263	126	76	50	146	0.61	1.15		
Subject B											
Basal l hour after	$\begin{array}{c} 613 \\ 822 \end{array}$	$\begin{array}{c} 164 \\ 428 \end{array}$	388 597	$\begin{array}{c} 125 \\ 133 \end{array}$	75 76	50 57	$\begin{array}{c} 274 \\ 210 \end{array}$	0·67 0·57	$2.19 \\ 1.57$		
2 hours after 3 hours after	 874	481	642	139	81	<del></del>	200	0.58	1.43		

healthy. Both lived at Dundas Harbour, on Devon Island (lat. 74° 35'), and had had practically no carbohydrate food other than the glycogen of animals for about 10 months before the tests. In each case, the test was commenced in the fasting state and the concentrations of the different plasma lipoids were determined before and again 1, 2 and 3 hours after administration of the oil.

It will be noted that there was a definite increase of neutral fat and of the ratio of phospholipins to total cholesterol in both cases. Both also showed a reduction of the concentration of phospholipins and of the ratio of ester to total cholesterol. The fatty acids increased in both cases, but not to the same extent. The ester cholesterol increased in one case and decreased in the other.

Interpretation of the above findings is difficult for a number of reasons. A survey of the literature shows wide variations of the different lipoid constituents of blood, both in fasting experiments and following ingestion of food, in animals and in man, and whether the analyses were made upon whole blood, red blood cells, plasma or serum. Correlation of these data is difficult because of the variety of technical methods with which they were obtained. With the same technique as used in our work, Boyd [1935] concluded that the concentrations of the different plasma lipoids were not consistently or markedly affected by intake of food. This, however, obviously applied to people on ordinary diets, with the ordinary mechanisms for the utilization of carbohydrate, fat and protein. That

the Eskimo differs from civilized peoples, at least with respect to tolerance of fat, is suggested from the findings of Tolstoi [1929] in two healthy men who lived for one year on lean and fat meats exclusively. In both of these subjects, these diets resulted in a definite increase of total fat and cholesterol; whereas, the average concentrations of these lipoids were not only lower than the standard in the group of Eskimos as a whole, but, also, lower in those natives whose diets consisted chiefly of meat (Table III).

Also suggestive of an unusual mechanism for the utilization of fat is the absence of ketosis in these natives, whereas the urines of both of Tolstoi's subjects contained acetone. The explanation of this absence of ketosis is not entirely clear. As shown previously [Rabinowitch & Smith, 1936], though the small amount of carbohydrates in the diets may be more than balanced by the potential sugar production from the large amount of protein to keep the ratio of fatty acid to glucose below the generally accepted level of ketogenesis, the respiratory quotient data suggest another mechanism also. That the Eskimo possesses a very active fat metabolism is suggested from some of the data. One of the theories of fat metabolism is that fat which has been ingested or mobilized from the fat stores is brought to the liver where it undergoes desaturation and transformation into highly unsaturated phospholipins and it is the latter which are carried to the tissues for oxidation. Assuming this theory to be correct, a very active fat metabolism is suggested from the high ratios of phospholipins to total cholesterol not only in the group as a whole (Table II) but also following ingestion of the soya bean oil (Table IV).

The low cholesterol values are of interest, since they fit in with the high basal metabolic rates which were found amongst these natives [Rabinowitch & Smith, 1936]. However, interpretation is difficult here also because of the many variables which had to be considered in the calculations of these rates. The chief purpose, therefore, of this communication is to record the facts for future use and better interpretation with accumulation of more knowledge of the metabolism of fat.

### Total protein content of plasma

An interesting metabolic finding amongst these Eskimos was the high concentrations of non-protein nitrogen in the bloods in the absence of albuminuria and evidence of disease of the kidneys clinically [Rabinowitch & Smith, 1936] and, apparently, due to the dietary habits of these natives. When food is plentiful, a healthy adult may eat 5 lb. or more of meat per day. It was, therefore, considered of interest to include in this study analyses of total protein contents of the bloods.

Method. It will be noted that the preliminary treatment of the blood samples and the subsequent extraction process for the lipoid analyses permitted determination of total protein concentrations in the same samples. The procedure was as follows. The precipitates obtained in the final filtration during the extraction of the lipoids were washed repeatedly with boiling 2% acetic acid and allowed to dry. The nitrogen contents of the residues so obtained, including the filter-papers, were then determined by the standard macro-Kjeldahl technique. Separate analyses were then made of the nitrogen contents of the filter-papers used in this work and the values obtained were taken into consideration in the calculation of the data. Control studies with other samples of plasma showed that this method was practically as exact as determination of total protein content of plasma by the usual methods.

Results. Of the 27 analyses, the total protein values ranged between 6.4 and 8.0% and no appreciable differences were noted between the meat-eaters and

those on mixed diets; the average concentrations of protein were 7.0 and 7.2% respectively. Therefore, in the Eskimo at least, habitual ingestion of enormous amounts of meat does not appear to result in increased concentration of protein in the plasma.

#### SUMMARY

A study was made of the concentrations of the different lipoid constituents of the blood plasma in 27 Canadian Eastern Arctic Eskimos.

The analyses included total lipoids, neutral fat, fatty acids, total, free and ester cholesterol and phospholipins. In two of the natives, the concentrations of these constituents were also determined before and after administration of 200 ml. soya-bean oil.

The combined data fit in with the respiratory quotients obtained from some of these Eskimos previously and suggest not only a very active but also an unusual mechanism for utilization of fats. The difficulties of interpreting these data are briefly discussed.

In the Eskimo, at least, it would appear that habitual ingestion of enormous amounts of meat does not lead to increased concentration of protein in the plasma.

The above studies were part of a general investigation of the health of Eskimos in Canada's Eastern Arctic. Space does not permit mention of the many whose co-operation made this investigation possible. Due acknowledgement was, however, made in the report to the Dominion Government and in a previous communication [Rabinowitch, 1936].

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